Research Article

RP-HPLC method development and validation for the estimation of Sofalcone in bulk drug and formulations with forced degradation studies

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Abstract

Objective: The current study was to develop a simple, accurate, precise, reproducible and economic reverse phase HPLC method for Quantification of sofalcone in bulk drug as well as in formulations. **Material and Methods:** The method development can be achieved by C 18 column (150x4.6 mm and 5µm particles) was used for the estimation of Sofalcone using 0.1 M Ammonium acetate buffer with 1 ml tiethylamine (pH 5.6) and acetonitrile in a ratio 50:50%v/v as mobile phase and at ambient temperature and the detector was set at wavelength 348nm. ICH guidelines were followed for method validation. Forced degradation studies were also done by exposing the drug to different stress conditions (Photolytic, thermal, acidic, alkaline, and Oxidative). The developed validated method was also utilized to quantify the Sofalcone in the marketed formulation successfully. **Results and conclusion**: The method was found linear within the range of 50-150 µg/mL with LOD of 0.028 µg/mL and LOQ of 0.087µg/mL whereas recovery was found within the range of 100.02% -100.55%. Method was found to be able to distinguish the parent drug from degraded products and quantify Sofalcone in Dosage form (100.91%). A linear, robust and economic RP-HPLC method was developed with LOD and LOQ within the good range which can be adopted for the routine analysis of Sofalcone in bulk drugs and formulations.

***Keywords**:* Sofalcone, HPLC, validation, stability

Introduction

Sofalcone is a potent gastro protective agent. It is shown to inhibit gastric mucosal injuries, prevent gastric ulceration and formation of acute mucosal lesions. This compound belongs to the class of organic compounds known as linear 1,3-diarylpropanoids. These are organic compounds with a structure based on a C6-C3-C6 skeleton, where the two benzene rings are not linked together (Wen et al 2007; Kohno et al., 1987; Yoshiyama et al., 2000).

Literature shows Bioanalytical method has been developed for the estimation of Sofalcone but to the best of our knowledge, there is no published chromatographic method for in bulk as well as in the dosage form. So, the present paper describes a simple, accurate and precise method for for the estimation of Sofalcone and an economic method has been developed by keeping the organic solvent at the minimum possible level, which will finally reduce the cost of the method (Isobe et al., 1985; Piotrowski et al., 1991).

Materials and methods

All the chemicals used were of AR grade. Solvents used are of HPLC grade and purchased from MERCK. The water used was distilled and deionised by using Millipore (ELIX) system.

Instrumentation

Shimadzu LC-2010C HT system, equipped with quaternary pump, auto sampler unit, online degasser, column oven and PDA detector (SPD-M20A) was used. All the data were processed and monitored using at LC SOLUTION software provided by Shimadzu.

Chromatographic conditions

C18 column (150x4.6mm and 5µm particles) (Agilent) was used as stationary phase. The mobile phase composed of two

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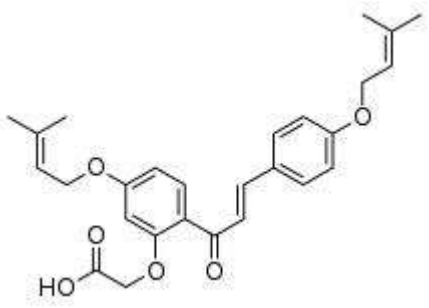

Figure 1. Chemical structure of Sofalcone

different components in the ratio 50:50 which were 0.1 M Ammonium Acetate buffer with 1 mL Triethylamine (pH 5.6- adjusted with Glacial Acetic acid) and Acetonitrile. The flow rate was optimized at 1 mL/minute and the injection volume was kept 5µl with a run time of 10 minutes. The chromatograms were recorded at 348 nm and column temperature was maintained at 25°C throughout the study period. Different samples prepared as well as mobile phase were filtered using 0.22µm filter and degassed by ultrasonication (LMUC 6) prior to use.

Preparation of standard stock solution

A stock solution of Sofalcone of concentration 100 µg/mL was prepared by dissolving 100 mg of Sofalcone in 5mL Tetrahydrofuran and make volume up to 100mL by using Diluent i.e. Water and Acetonitrile (20: 80) in a volumetric flask. Thereafter solutions of different concentration (50- 150µg/mL) were prepared by diluting the stock solution.

System suitability

It was determined by giving the six injections of the same concentration (100µg/mL) applying the chromatographic conditions of the proposed method. Thereafter %RSD of retention time, peak area, peak asymmetry, and theoretical plate were calculated.

Solution stability

For solution stability chromatograms of the same concentration were recorded at different time intervals (3, 9, 12, and 24 hours) and changes in RT, peak area, peak asymmetry and theoretical plates were observed.

Method validation

Different parameters i.e. Linearity, range, system suitability, specificity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ), Robustness and Solution Stability were studied for the validation of the developed method and method was validated as per the ICH guidelines (ICH guideline 2005).

Linearity and Range

Stock solution of concentration 100µg/mL was diluted to get the different concentrations 50 µg/mL, 80 µg/mL, 100µg/mL, 120 µg/mL and 150 µg/mL and 5µl of each concentration was then injected using auto sampler unit and the chromatograms were recorded. A graph of area under curve vs. concentration was then plotted to get calibration curve. The equation, slope, intercept and regression coefficient (R^2) were determined.

Precision

Three different concentrations were selected for Interday and intraday precision studies which are 50 µg/mL, 100µg/mL and 150 µg/mL. Three responses of each concentration were recorded by injecting 5 µl of sample on a single day for intraday whereas for interday on three different days. %RSD of sofalcone was found to be 0.3332 %, which is within the acceptable criteria (<2%).

Accuracy

In a pre-quantified solution of drug a known amount of drug 50mg, 100mg and 150mg were added and chromatogram was recorded as per the optimized chromatographic conditions. The percentage recovery was then calculated by fitting the area of the sample in the calibration curve equation.

Limit of detection (lod) and limit of quantification (loq)

As per ICH guidelines following equations were used to determine the LOD and LOQ

$LOD = 3.3 \times a/S$

$LOQ = 10 \times a/S$

Where a is standard deviation of y intercepts and S is the average of slope (Han et al., 2005).

Robustness

Deliberate changes chromatographic conditions like temperature, wavelength and instrument were made. The effect of these deliberate variations was studied on retention time, plate count and peak asymmetry parameters of the developed method. Changes were made in temperature conditions, wavelength and flow rate. Retention time, plate count and peak asymmetry parameters were observed.

Analysis of marketed formulation

A marketed formulation of Sofalcone capsule (Sofalco) was procured from local pharmacy. The tablet was crushed and it was diluted to get a solution equivalent to 100 µg/mL of Sofalcone and was analyzed using the optimized chromatographic conditions.

The chromatogram was also observed for the unwanted peaks due to excipients present in the formulation, at the

optimized Retention Time, to confirm the specificity of the method

Forced degradation studies

Alkaline and acidic degradation studies: Capsule powder equivalent to 100 mg of Sofalcone (2 Capsule) was transferred in 100 mL volumetric flask. To it 1 mL of 1N HCl was added and kept for 1 hour at room temperature. After that neutralization was done by using 1mL of 1N NaOH. Make up volume with diluent, sonicate it for 10 min then centrifuge it for 25min at 5000 rpm and then filter by using syringe filter and further diluted with taking 1 mL from the above solution and up to 10mL with diluent (Same procedure carried out for condition of 1 mL of 1 N HCL for 3 hour room temperature).

Oxidative degradation studies: Capsule powder equivalent to 100 mg of Sofalcone (2 Capsule) was transferred in 100 mL volumetric flask. 1 mL of 3 % H2O2 was added and kept for 25 min at room temperature. Make up volume with diluent, sonicate it for 10 min then centrifuge it for 25min at 5000 rpm and then filter by using syringe filter and further diluted with taking 1 mL from the above solution and up to 10mL with diluent.

Thermal degradation studies: Amount 100 mg of drug was kept in oven at 80°C for 3 hour. The drug was then properly diluted to give a final concentration of 100µg/mL. The chromatograms were run by injecting the sample in the column.

Photolytic degradation studies: 100 mg of drug was dissolved in 10 mL of Millipore water. The solutions were kept in the UV light for 3 h. The drug was then properly diluted to reach a final concentration of 100 µg/mL and analyzed by the optimized HPLC method (Wang et al., 2011).

Results and discussion

Linearity and range

The proposed method was found linear in the range of 50 µg/mL-150 µg/mL with a regression coefficient of 1.0000 and the regression line was having a slope of 63,041.76 and y-intercept 43,862.87 (equation y = 63,041.76x + 43,862.87) (Table 1; Figure 2(i) and 2(ii))

Precision

In precision studies the area values were obtained for both intraday and interday precision. The % RSD of three concentrations viz. 50 µg/mL, 100 µg/mL and 150 µg/mL, for intraday were 0.98, 0.69 and 0.60 while it was 0.46, 0.81 and 1.09 for interday studies. All the three concentrations were found with no significant change as the values of % RSD was within the limit (<2%) [Table 2].

Accuracy

In this study 50%, 100% and 150% of 100 µg/mL solution was added to the 100 µg/mL and analyzed by the proposed method and the % RSD for these three added concentrations, found were 0.0344 %, 0.0251% and 0.0151% respectively.

The method was found accurate as good recoveries (100.02-100.55%) were obtained for various added concentrations (Table 3).

Limit of detection (LOD) and limit of quantification (LOQ)

Table 1. Linearity and Range parameters

Sr.	Parameters	Results
1	Linearity (range) (µg/mL)	50-150
2	Retention time (min)	4.7
3	Regression coefficient (r^2)	0.9998
4	Slope	18.672
5	Intercept	1.8119
6	Equation	y = 18.672x - 1.8119

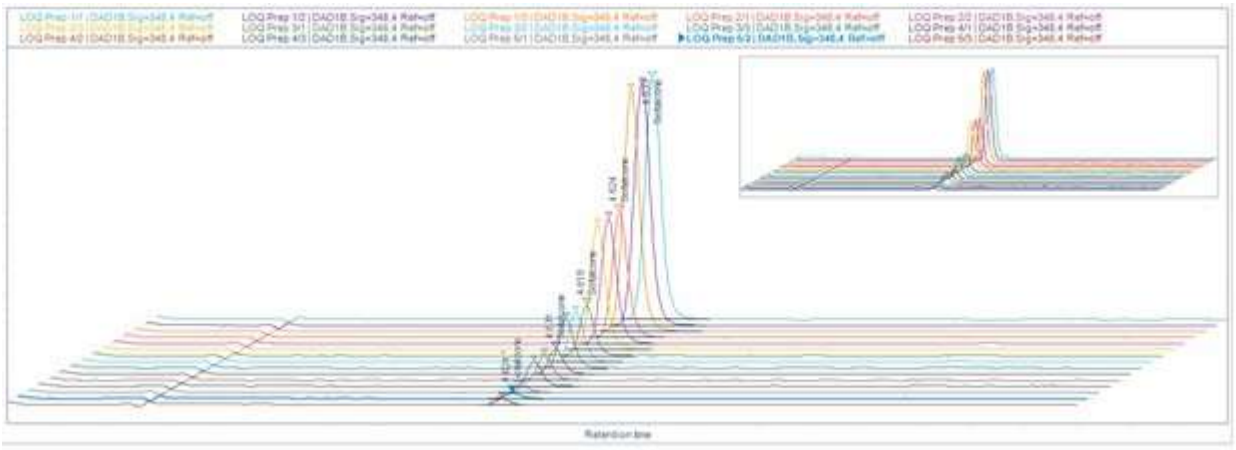

Figure 2. (i) Standard plot of Sofalcone (ii) Overlay chromatogram of Sofalcone [(a) 50µg/mL (b) 80 µg/mL (c) 100 µg/mL (d) 120µg/mL (e) 150µg/mL]

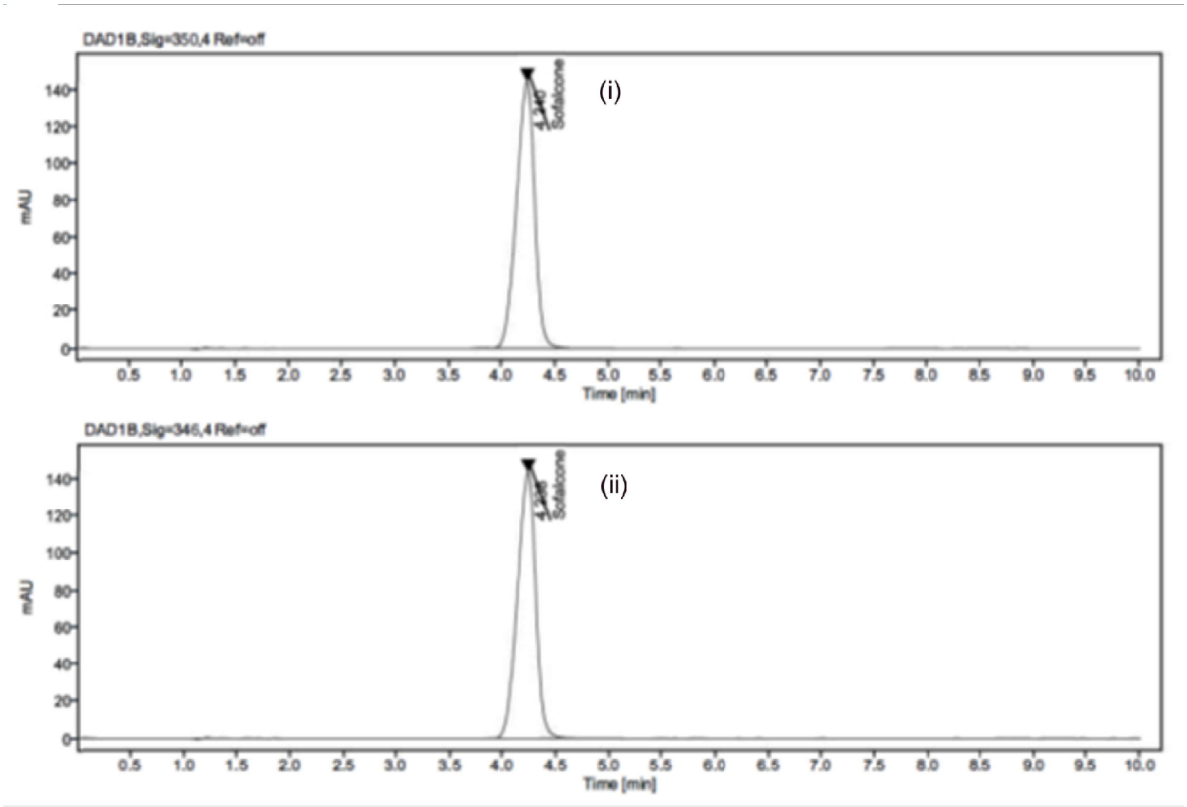

Figure 3. (i) Chromatogram at 350nm, (ii) Chromatogram at 346 nm

Table 2. Intraday and Interday Precision parameters

Inter-day (n=3)			Intra-day (n=3)		
Conc. (µg/mL)	**Mean ± SD**	**%RSD**	**Conc. (µg/mL)**	**Mean ± SD**	**%RSD**
50	100.12±0.98	0.98	50	99.43± 0.45	0.46
100	99.69 ± 0.69	0.69	100	100.39 ± 0.82	0.81
150	99.88± 0.60	0.60	150	99.94 ± 1.09	1.09

Table 3. Accuracy parameter of validation for three different concentrations

Sr. no.	(%) Spiked	Conc. from formulation	Standard Conc. Added	Conc. Recovered	%recovery	% RSD
1	50%	100	50	50.20	100.40	0.0344
2	50%	100	50	50.22	100.44	
3	50%	100	50	50.25	100.50	
4	100 %	100	100	100.04	100.04	0.0251
5	100 %	100	100	100.07	100.07	
6	100 %	100	100	100.02	100.02	
7	150 %	100	150	150.78	100.52	0.0151
8	150 %	100	150	150.81	100.54	
9	150 %	100	150	150.82	100.55	

LOD and LOQ were found to be 0.028µg/mL and 0.087µg/mL respectively for sofalcone, that means method is able to detect and quantify small amount of drug (Table 4).

Robustness

Wavelength (± 2nm): Deliberate changes were made in wavelength (± 2nm) and sample was analyzed at 350nm and 346nm (Figure 3(i) and figure 3(ii)). The values obtained for sample analyzed at 348nm for retention time (RT), Plate count (PC) and peak asymmetry (PA) were found 4.681, 1896.28, 0.87 respectively while the results for sample analyzed at 348nm were found 4.685, 1858.04 and 0.83 respectively.

Temperature (± 5°C): The current method was optimized

Table 4. Limit of Detection (LOD) & Limit Of Quantification (LOQ)

Parameters	Value
Standard deviation of intercept	880.241
Slope mean	100172.44
Limit of detection	0.028 µg/ml
Limit of quantification	0.087 µg/ml

Table 5. Wavelength, Temperature & Flow Rate Parameters for Robustness Studies

Parameters	Optimized	Used	Retention Time (RT), Min	Plate Count	Peak Asymmetry
Wavelength (± 2nm)	348nm	350	4.681	1896.28	0.87
		346	4.685	1858.04	0.83
Temperature (± 1 ^{o}C)	25^{o}C	20^{o}C	4.693	1889.18	0.88
		30^{o}C	4.630	1889.63	0.85
Flow rate (mL/min)	1.0	0.9	4.238	1719.87	0.88
		1.1	5.131	2097.64	0.85

Table 6. Solution Stability parameter

Conc. Used(µg/mL)	Mean area	SD	%RSD
100	1898.71	18146.5	0.284309

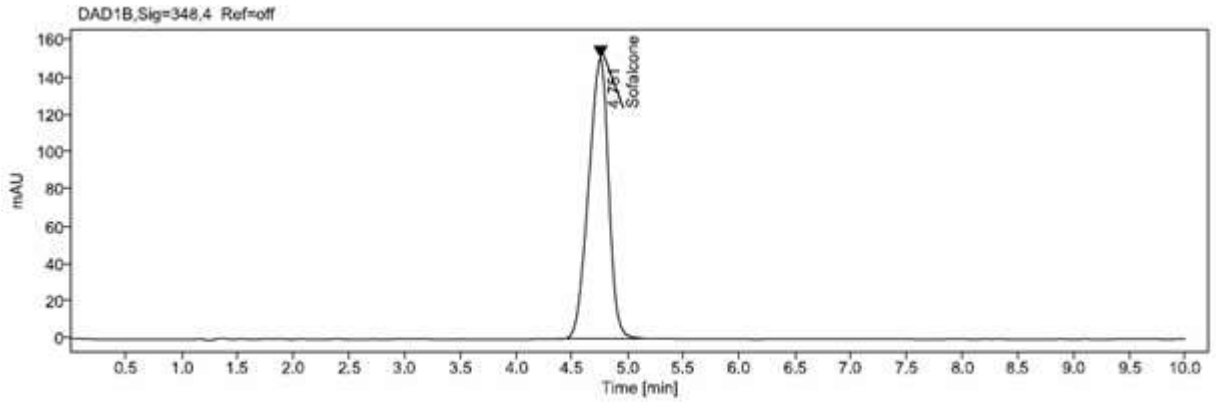

Figure 4. Chromatogram of marketed formulation with optimized chromatographic conditions

at 25°C and to study temperature robustness samples were analyzed at 20°C and 30°C and results obtained for both temperatures for RT, PC and PA were found to be 6.722, 15429.12, 1.449 and 6.769, 15562.5, 1.446 respectively.

Flow rate (± 0.1): The flow rate was optimized at 1 mL/minute and to study temperature robustness samples were analyzed at 0.9 mL/minute and 1.1 mL/minute and results obtained for both Flow rate for RT, PC and PA were found to be 4.238, 1719.87, 0.88 and 5.131, 2097.64, 0.85 respectively.

Deliberate changes were made in wavelength (± 2nm), temperature (±1°C) and instrument and it was found that all the parameters were within the prescribed limit i.e. plate count was found >4000 and tailing factor was found <2 but changing in flow rate change the retention time of main peak (Table 5).

Solution stability

100µg/mL concentration solution was used for solution stability studies and the mean area was found to be 1898.71 with % RSD 0.284309mL and there is no significant change was observed in the peak area (RSD <2%) (Table 6).

System suitability

For system suitability six injections of 100µg/mL concentration were injected and responses were observed for Rt, peak area, tailing factor and theoretical plates, the values for which are 4.7468, 1893.208, 0.884, 3160.2 respectively and %RSD for all these parameters were found to be 0.1176, 1.4496, 1.01179, 1.1190 respectively.

For system suitability six injections of 20µg/mL

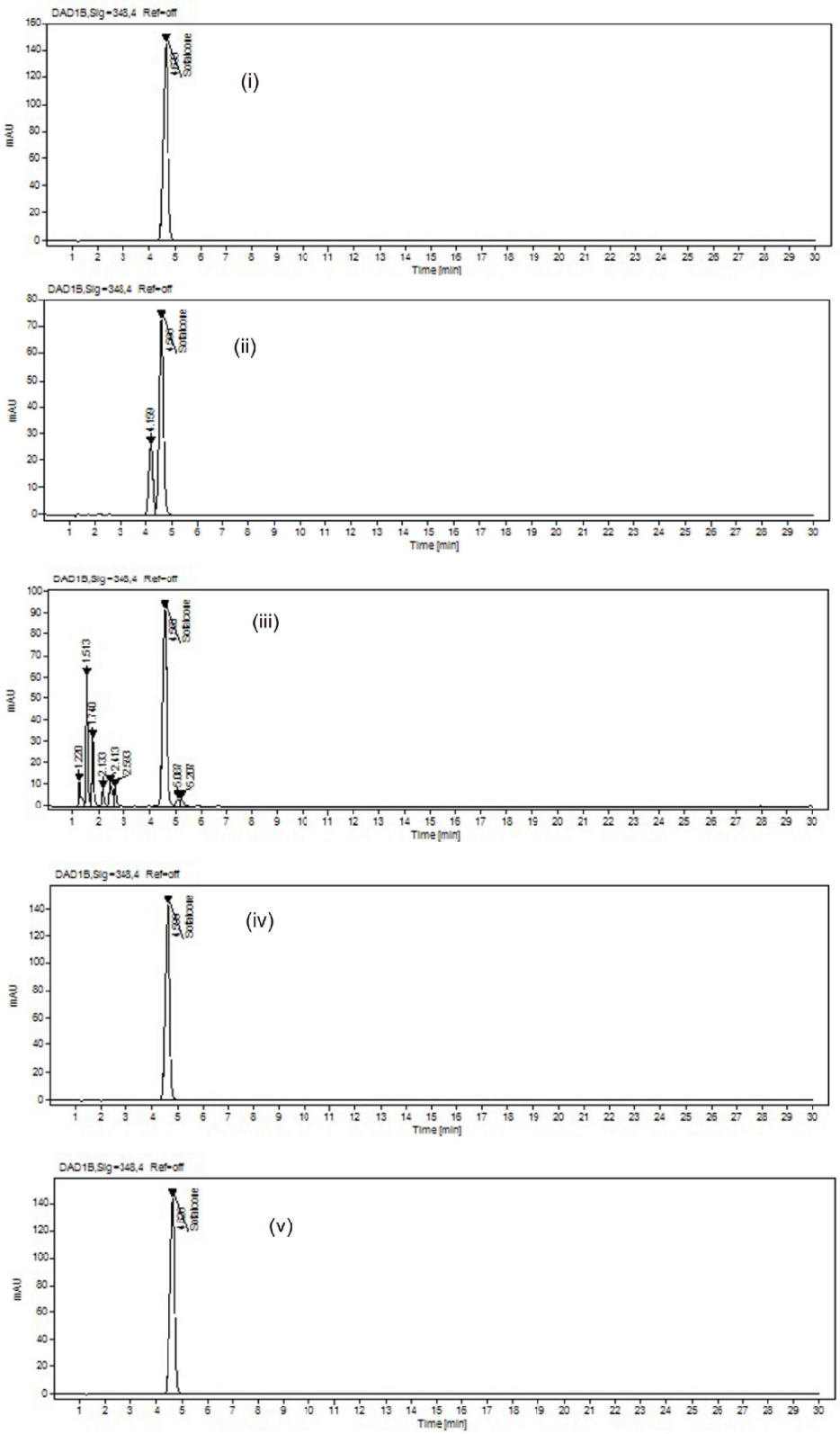

Figure 5 (i) Chromatogram after thermal degradation; (ii) Chromatogram after photolytic degradation; (iii) Chromatogram after acidic degradation; (iv) Chromatogram after alkaline degradation; (v) Chromatogram after oxidative degradation

concentration were injected and responses were observed for RT, peak area and Tailing Factor and all were found within the limit (RSD <2%) (Table 7).

Analysis of marketed formulation

Solution of marketed formulation (concentration 100µg/mL) was injected at the optimized chromatographic conditions and

Table 7 System Suitability parameters

Parameters	Observed results ± S.D	% RSD	Acceptance criteria
Peak Area	1893.208±27.4457	1.44%	%RSD<2
Retention time (RT)	4.74±0.0055	0.11%	%RSD<2
Theoretical plates (N)	3160.2±35.3652	1.11%	> 2000
Tailing factors (T)	0.884±0.0089	1.01%	T< l.5

Table 8 Analysis of Marketed Formulation

Formulation	Labeled amount (mg)	Amount found (mg)	%Label claim ± SD Assay (n=3)	%RSD
Sofalco	100	100.25 100.75 101.75	100.91 ± 0.763	0.756

100.91 % recovery was obtained (Table 8; Figure 4).

Forced degradation studies

An ideal stability indicating method is the one which is able to differentiate the degradation products and at the same time must be able to quantify the drug. In the present study, the drug was forced to degrade under oxidative, acidic, alkaline, photolytic and thermal stress condition. After the forced degradation when drug was analyzed by the optimized method it was observed that the there was no degradation during thermal, alkaline and oxidative stress conditions. On the other hand in case of Acidic stress conditions peaks of 6 degradation products at different retention times along with the peak of API at 4.6 were found. Also under photolytic stress conditions there was 1 degradation product peak and the chromatogram showed the peak of parent drug [Figure 5(i) to 5(v)]. From the result it was found that Sofalcone was degraded 44.1% in acid degradation and 56.08% in photolytic condition.

Conclusion

In the present study a RP-HPLC method has been described for the quantitative determination of Sofalcone in bulk drug as well as in the formulation. The method has been validated for different parameters like linearity range, system suitability, specificity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ), Robustness and Solution Stability and the results obtained were found statistically significant. Degradation of drug substances between 5 and 20% has been accepted for validation of chromatographic assays. From the results, it was found that sofalcone was degraded significantly by acidic and photolytic stress condition. That is why we chose the pH of buffer in basic region and close to neutral pH. This method is economic due to very less usage of organic content in the mobile phase and hence recommended for the routine quantitative analysis in the pharmaceutical industries.

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